Permutation Achieved Classification Error (PACE): A Significance Test for Assessing the Statistical Significance of Classifiers for Peptide Profiling via MALDI/SELDI-TOF Mass Spectrometry

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CS Department Faculty Recruitment Funds (MH)

Outline

- PACE Analysis
- Pancreatic Cancer Preliminary Classifier
- Negative Results
- Published Data
- Caveats
- Biological Significance
- Parting Shots

Published SN and SP from SELDI-TOF-MS Studies

- Ovarian Cancer:100%, 95% **(Liotta et al., 2002)**
- Prostate Cancer: 100%, 100% **(Qu et al., 2003)**
- Breast Cancer: 90%, 93% **(Vlahou et al., 2003)**
- Breast Cancer: 91%, 93% **(Li et al., 2002)**
- Head & Neck: 83.3%, 90% **(Wadsworth et al., 2004)**
- Lung Cancer: 93.3%, 96.7% **(Xiao et al., 2003)**
- Pancreatic Cancer: 78%, 97% **(Koopmann et al., 2004)**

Are these REAL?

- Diamandis
- Baggerly et al.
- New York Times…
- Etc…

Dr. Diamandis

- Diamandis EP. 2004. Analysis of serum proteomic patterns for early cancer diagnosis: drawing attention to potential problems. J Natl Cancer Inst. 96(5):353-6.

- Diamandis EP. 2004. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool: Opportunities and potential limitations. Mol Cell Proteomics. 2004 Feb 28. [Epub ahead of reprint]

- Diamandis EP. 2004. Re: diagnostic potential of serum proteomic patterns in prostate cancer. J Urol. 171(3):1244-5; author reply 124-5-64.

- Diamandis EP. 2004. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool: Opportunities and potential limitations. Mol Cell Proteomics. 2004 Jan 30 [Epub ahead of print]

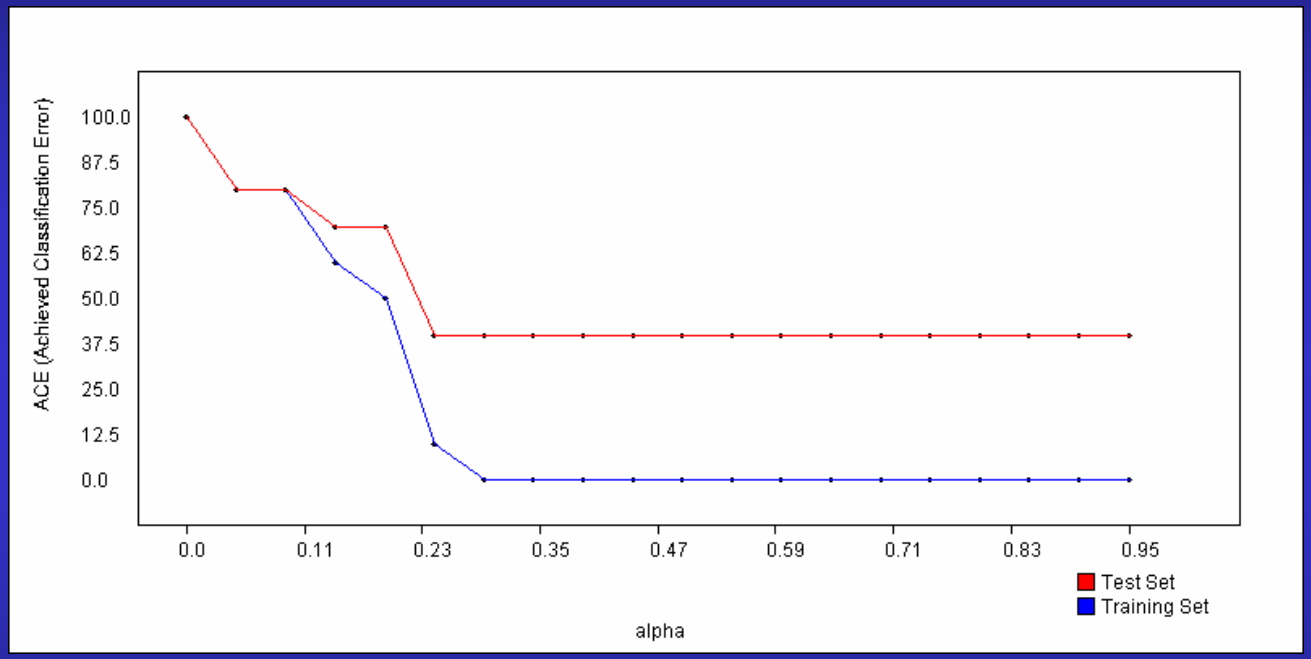
Practical significance of some of these concerns

- Overtraining (model overfit)
- Biased estimate of Achieved Classification Error (ACE)
- Low External Generalizability of Model

What is Overtraining?

ACE

Random Resampling
N1=N2=8
5000 random features



stringency of feature selection method

Bias in ACE

- **A**chieved **C**lassification **E**rror = the proportion of cases that are incorrectly predicted.
- High biased estimates can be either too optimistic *or* pessimistic (conservative)
- Use of TEST cases protects against this bias
- Various learning evaluation schemes provide low-biased esitmates of ACE in general population:
  - Leave-one-out Validation
  - *k*-fold Validation
  - Hold-out m% method
  - Random Resampling hold-out method

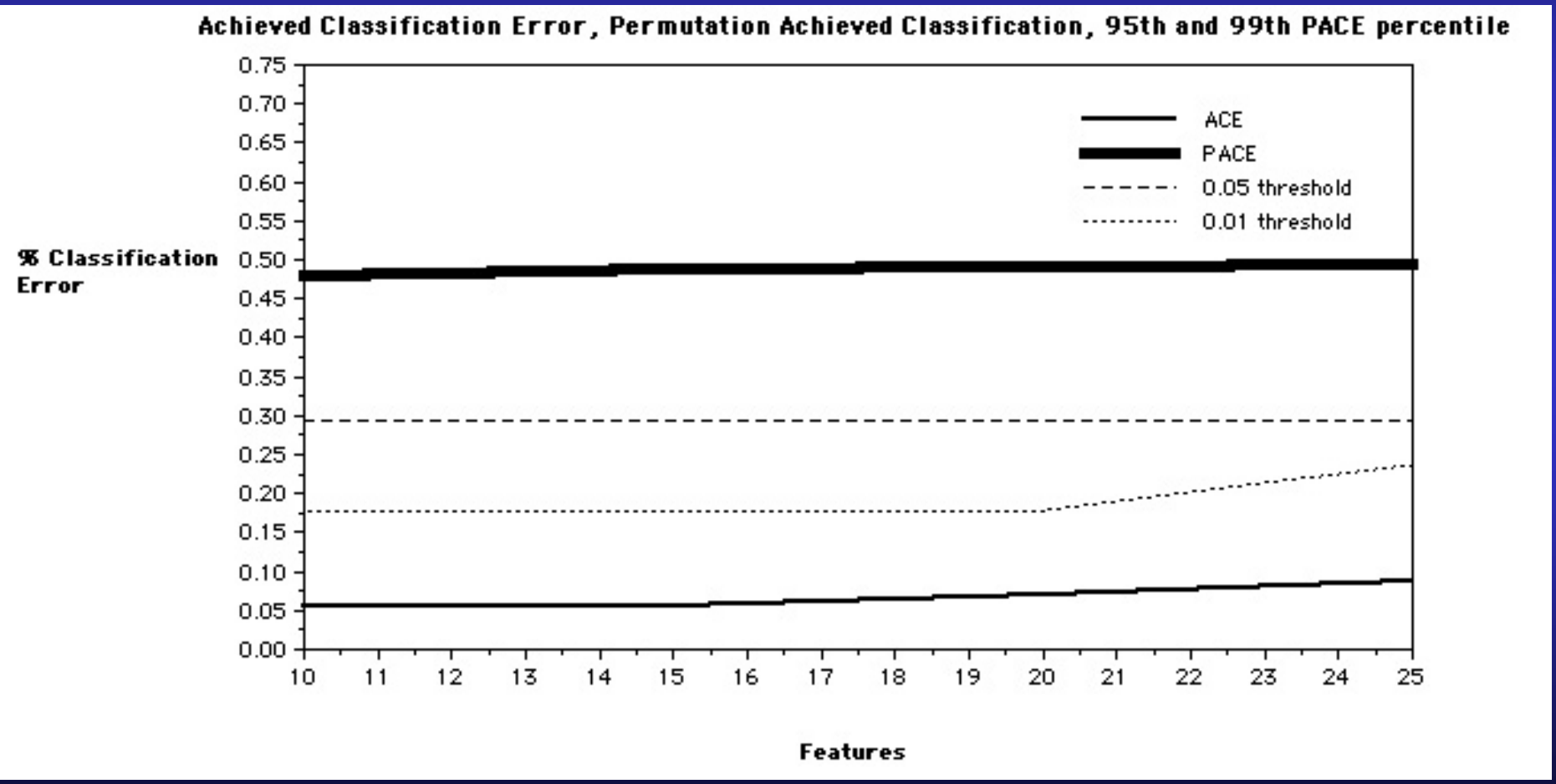
PACE: Permutation Achieved Classification Error

- Expected classification error under the null model (one homogeneous population, stochastic variation in profiles reflect random differences only, no signal)
- This is 50% (a coin toss) in case vs. control studies

Approach

- Determine ACE using a method x
- Create 1,000 null data sets via random sample class labeling
- Perform analysis on all 1,000 null data sets
- Determine Mean Achieved Classification Error (MACE) and
- 95th, 99th Percentile of the PACE distribution
- Compare ACE to PACE distribution

Pancreatic Cancer Result



Adenocarcinoma of the Pancreas

- 30,300 cases in the U.S. in 2002
- Ninth most common cancer but…fourth leading cause of cancer deaths (5-6 % of all cancer-related deaths)
- Overall 5-year survival is <5%
- For the minority of patients with resectable disease

  5-year survival is ~20%, however 80% of these patients will recur within 2 years and die of their disease

Survival after Resection of Adenocarcinoma of the Pancreas

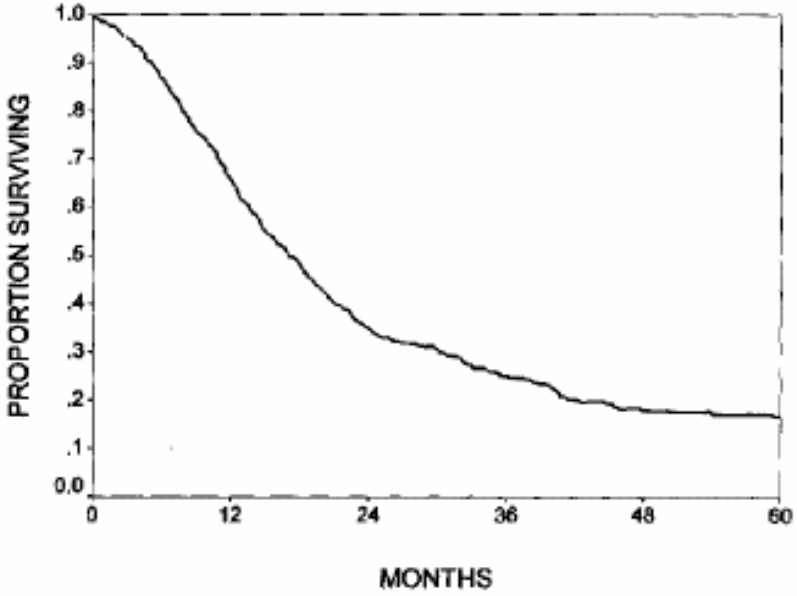
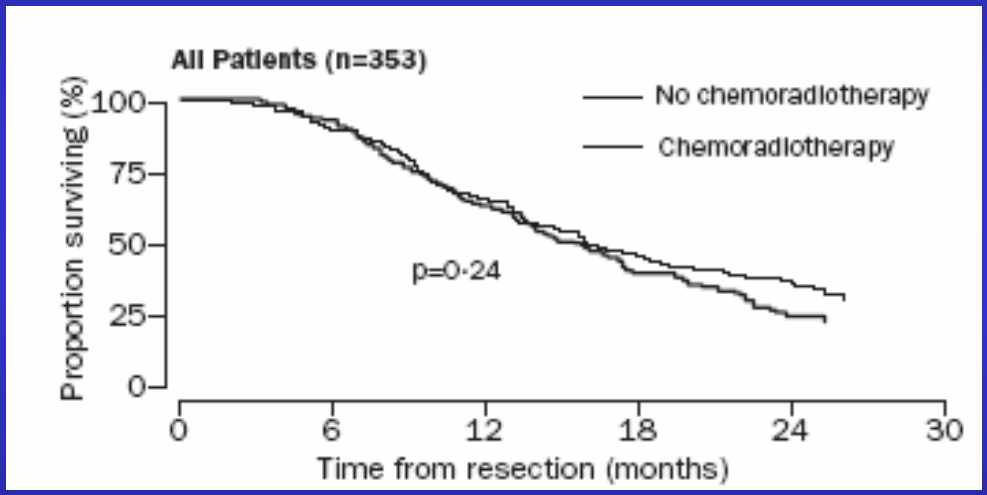


Fig. 1. Kaplan-Meier actuarial survival for entire cohort of 616 patients with resected pancreatic adenocarcinoma.

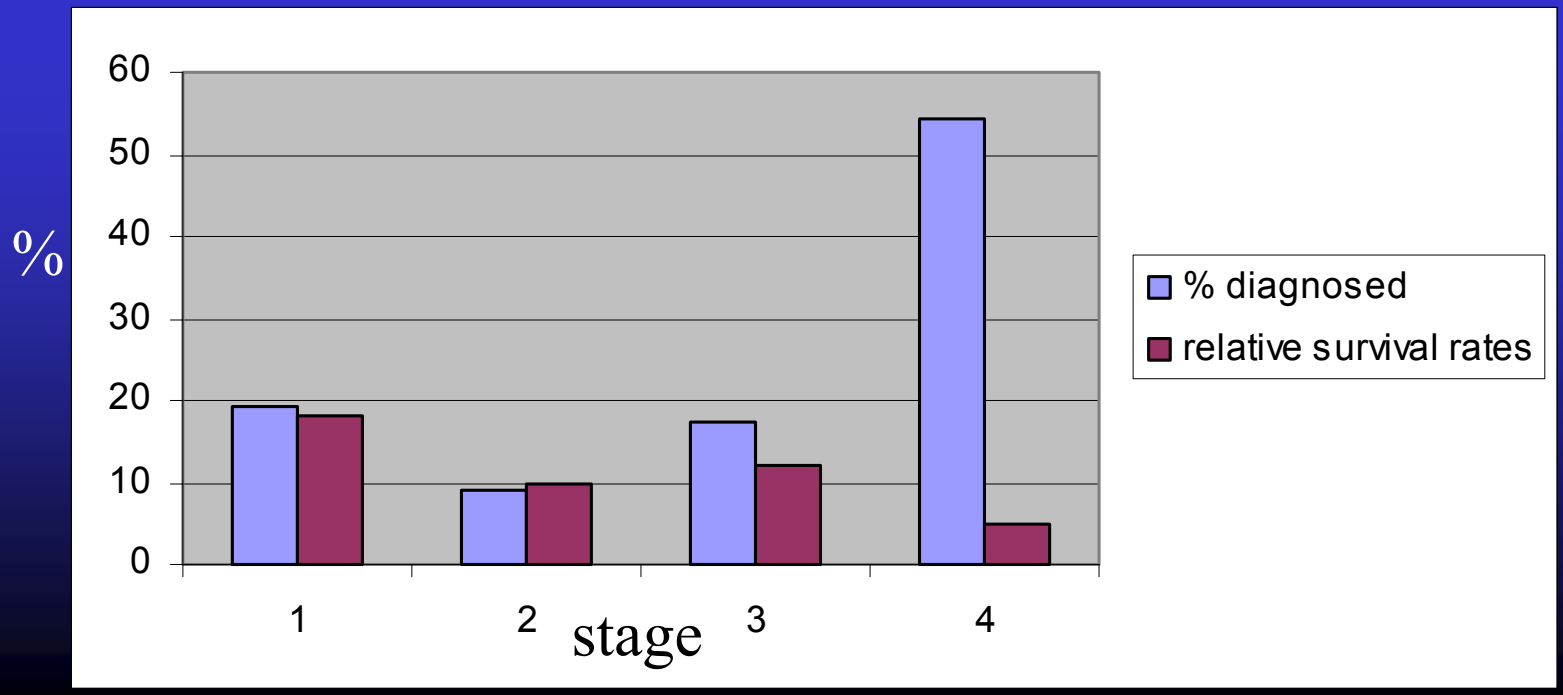


Neoptolemos et al., 2001 *Lancet*

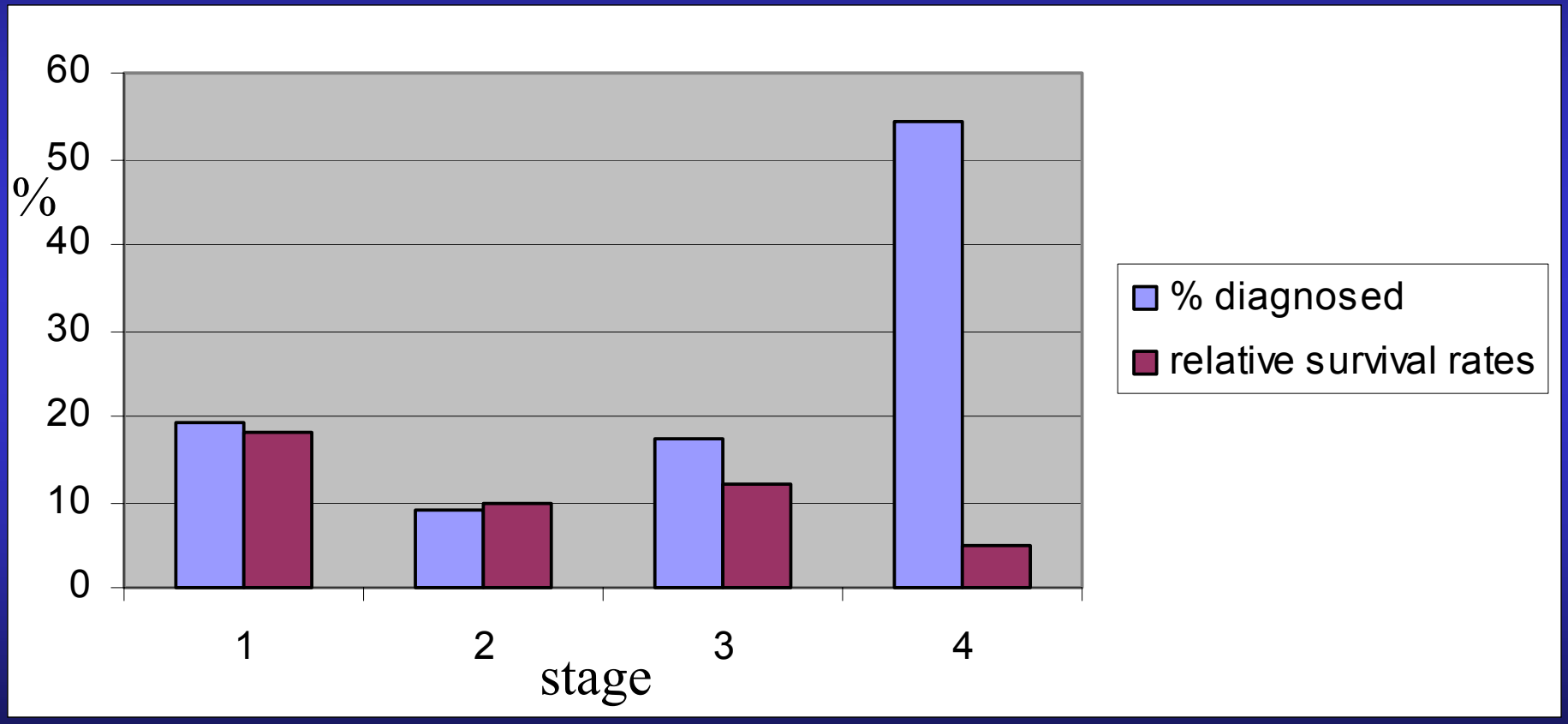
Sohn *et al* Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. *J Gastrointestinal Surgery* **4**: 567-579, 2000.

Neoptolemos JP, et al. Ann Oncol. 2003 May;14(5):675-92.

- Overall median survival from diagnosis < 3-5 mos
- 12-mo survival rate of ~ 10%
- 5 – year survival rate of 0.4-3%



Pancreatic Cancer Stage at Diagnosis and Survival



NCDB Highlights, 1996

Retrospective data support the concept that early detection and treatment will lead to improved survival in patients with pancreatic cancer

- Patients with smaller tumors had better overall survival, < 3.0 cm ($P = 0.004$)
- Survival of a select group (N = 75) of patients with small tumors (< 3.0 cm), negative margins, and negative lymph nodes:
  - 1 Y - 81%
  - 3 Y - 46%
  - 5 Y - 31%

Sohn *et al* Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. *J Gastrointestinal Surgery* **4**: 567-579, 2000.

Ariyama *et al* 1990. *Int J Pancreatol. 7:37-47.*

- Reported 100% 5-year survival in patients undergoing resection of pancreatic tumors detected very early (<1 cm)

Risk of cancer	Specificity/Sensitivity	Years gained
5%	0.80	0.11
10%	0.80	0.49
20%	0.80	1.24
30%	0.80	1.98
5%	0.90	0.28
10%	0.90	0.69
20%	0.90	1.52
30%	0.90	2.34

Kern S,H.R.H.M. 2001. **A white paper: the product of a pancreas cancer think tank**. *Cancer Res* 61:4923-4932.

Available Serum Tumor Biomarkers for Pancreatic Cancer

- CA19-9 levels are correlated with the presence of pancreatic cancer
  - Sensitivity and specificity for CA19-9 between 70-90% and 90%, respectively
  - Unfortunately, there is a high degree of overlap between pancreatic cancer and a variety of benign inflammatory conditions of the pancreas, limiting the clinical applicability of CA19-9 as a specific early detection/screening marker
- Tissue polypeptide specific antigen (TPS) (a breakdown product of the extra-cellular matrix) is another recently described serum tumor marker that has been reported to provide improved discrimination between pancreatitis and pancreatic cancer over CA19-9
  - Sensitivity and specificity of 97% and 98%, respecitvely vs versus normal controls
  - However, TPS was also found to be elevated in 17-20% of patients with chronic pancreatitis

Study Design and Subject Accrual

- Study Design and Target Accural
  - 30 newly-diagnosed pancreatic cancer cases,
  - 30 case-matched healthy controls,
  - 30 chronic pancreatitis

- Currently Accrued
  - 120 cases since October 2002,
  - 80 pancreatic and 40 non pancreatic “Whipple controls”,
  - Over 100 acute and chronic pancreatitis,
  - Over 100 matched controls

Patients and Controls (see Herb)

- Preoperative serum samples from 32 pancreatic cancer cases (17 female, 15 male)
- Patient samples were obtained in the operative room using fast-stabilizing protocols to protect against trypsin (Whitcomb, MD, PhD)
- 23 non-cancer age-, gender-, and smoking history-matched controls were analyzed.
- Ages ranges 34-87; mean age PCa 64; controls 67 ($p=0.19$)
- 16 were resected; 6 patients had locally advanced unresectable disease, 10 had metastatic disease.

SELDI-TOF-MS Profiling (see Dave)

- The serum samples were denatured and processed in duplicate on a single type (IMAC3-Cu) ProteinChip® Array (Ciphergen Biosystems, Fremont CA).
- Samples were processed in random order to avoid confounding sample type with temporally autocorrelated laboratory conditions
- Biomek2000 liquid-handling robotic workstation (Beckman Instruments, Inc., Fullerton CA).
- Whole serum samples ('neat spotting')
- ProteinChips were read in a PBSIIc mass spectrometer (Ciphergen) using positive ion mode, with time delay focusing, from 0-100 kDa.
- Mass calibration was performed externally, using a mixture of seven peptide species from 1-7 kDa (Ciphergen).
- Spectra were preprocessed by baseline subtraction with smoothing; filtering (averaging, by 0.2x expected peak width) and normalized by total ion current.

Analysis

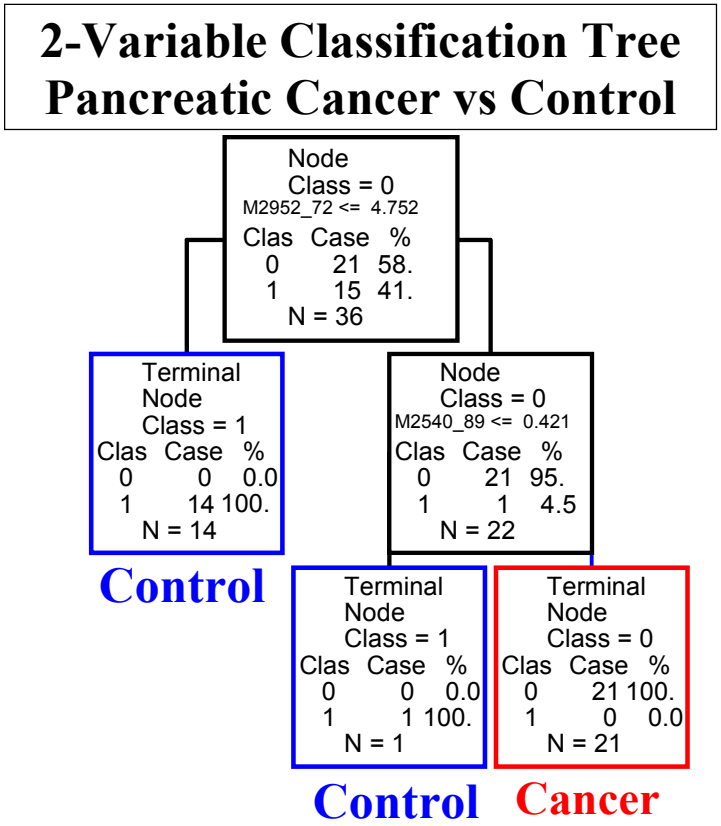
- CART Analysis (Ciphergen BPS software; Dave)
- MatLab functions developed by Milos Hauskrecht, Richard Pelikan, CS Department, JL-W
- Feature Selection
  - Fisher Score, w, w/out Decorrelation
  - Principal Components Analysis (aggregate feature)
- Classification
  - Naïve Bayes
  - Support Vector Machine (SVM)

CART

- ‘training set’ of 21 cases/15 controls,
- ‘testing set’ of 11 cases/8 controls.

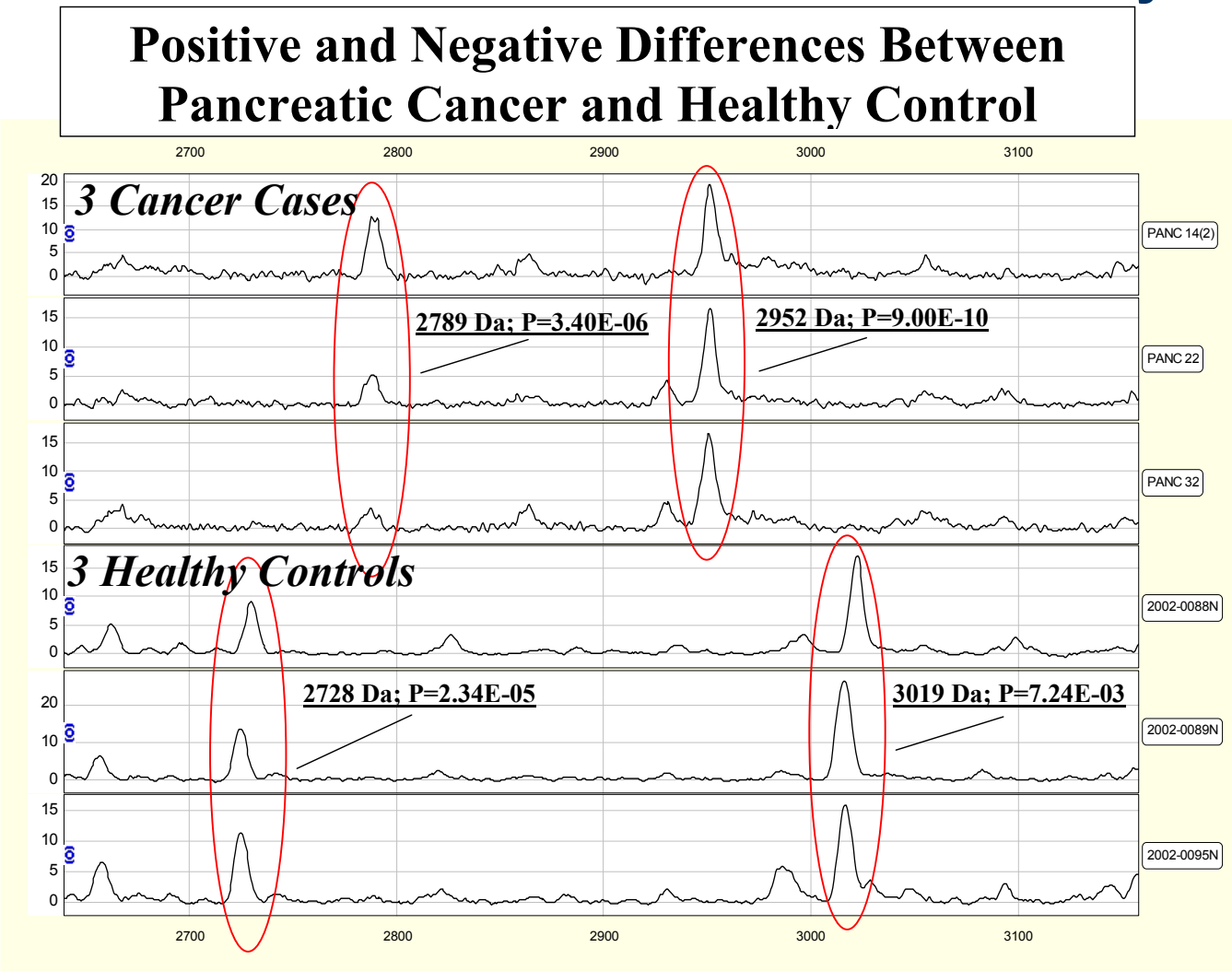
- 64 most significant peaks from a pair-wise comparison using CART analysis (Ciphergen Biomarker Patterns® Software).

SELDI-TOF MS Serum Profiling Discriminates Pancreatic Cancer Cases from Matched Healthy Controls



Training Data:	Testing Data:
100% Sens (21/21)	91% Sens (10/11)
100% Spec (15/15)	100% Spec (8/8)

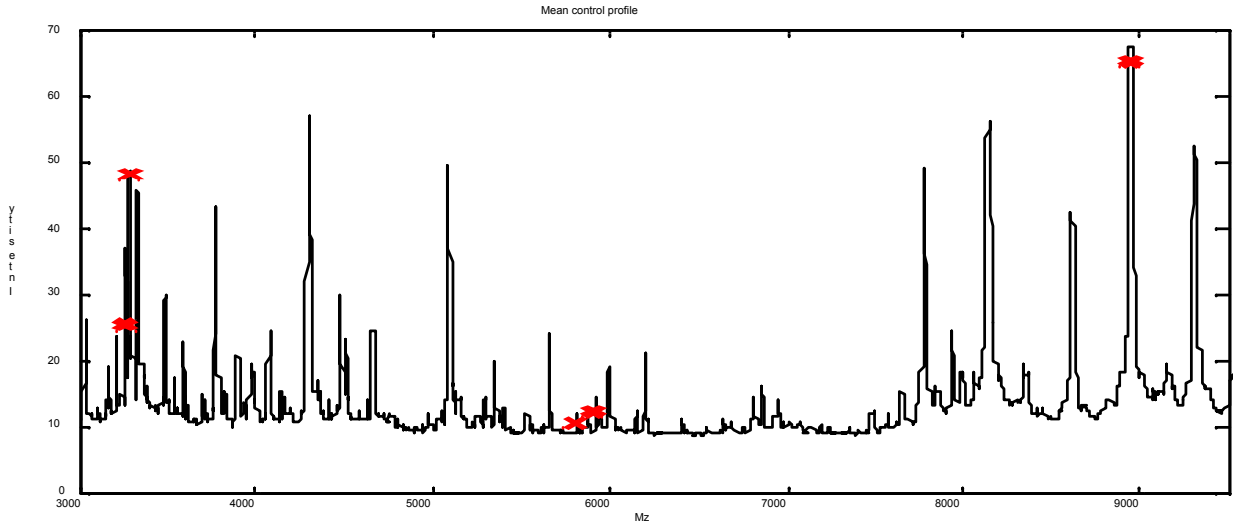
SELDI-TOF MS Serum Profiling Discriminates Pancreatic Cancer Cases from Matched Healthy Controls

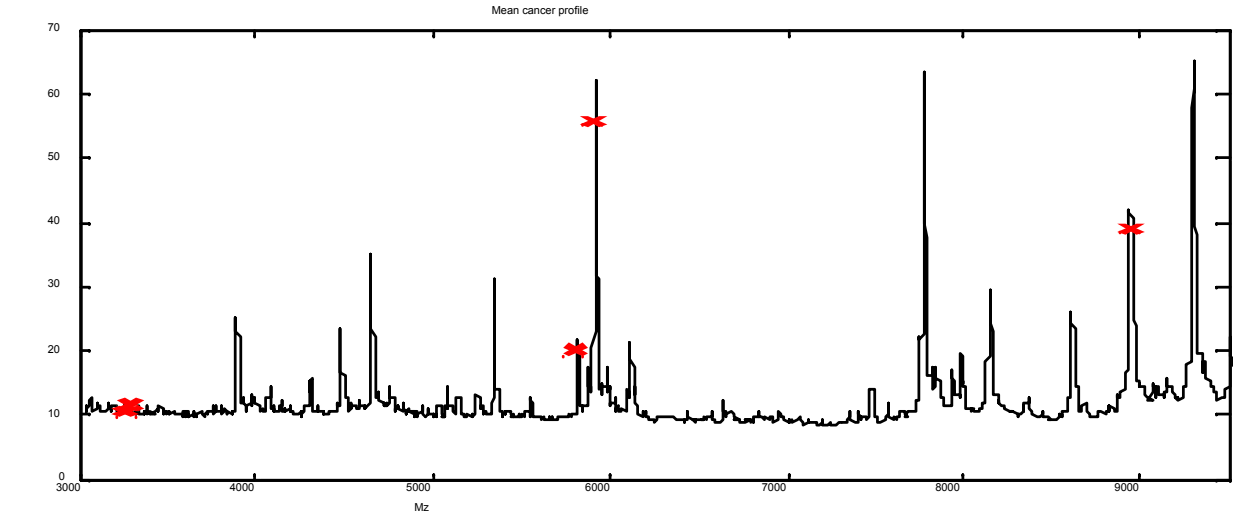


Supervised Learning

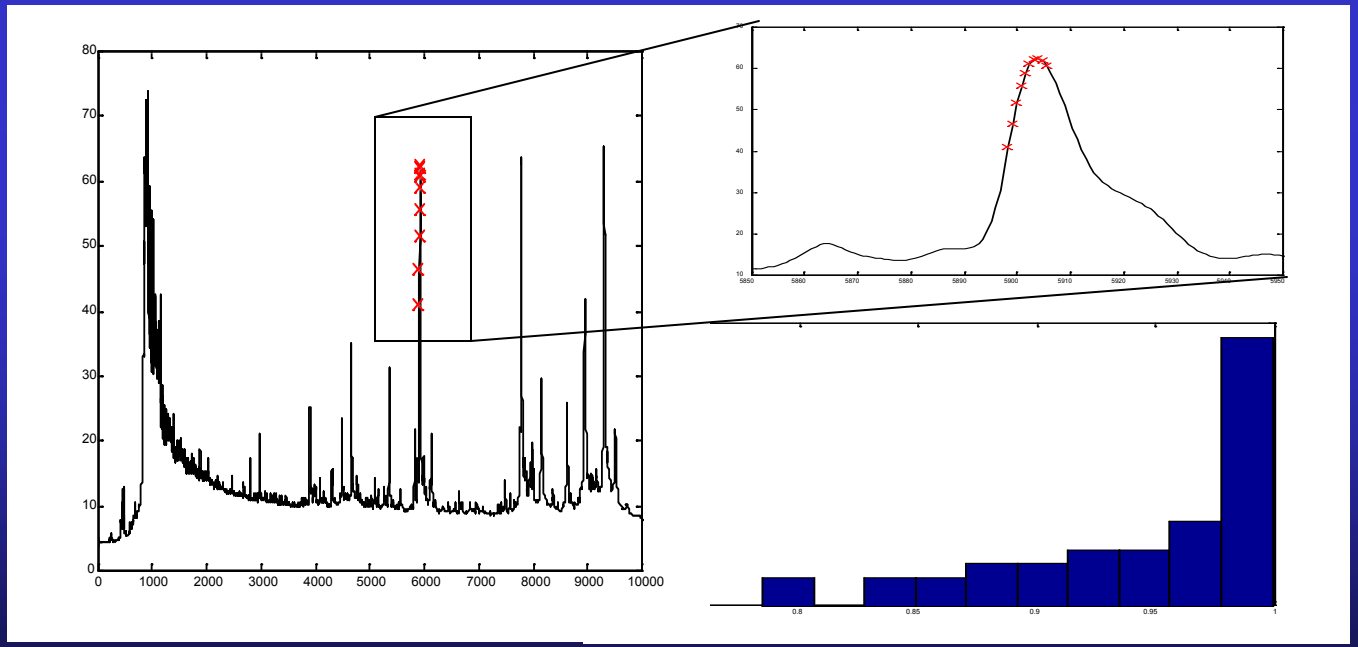
- Post-Feature Identification
- Find Informative Features
  - "Differentially expressed features"
    - t-test, modifed t-test (e.g., SAM), AUC, Fisher score
    - Decorrelation filtering (Décor)
  - Aggregate Features (PCA)
- Predict Class Labels
  - Classification predictions via Naïve Bayes or linear SVM
- See Milos, Richard or poster for details…

Mean Cancer and Control Profiles (Top 5 Fisher Score Positions)





- Decorrelation after feature ranking is similar to PCA
- Useful because the most significant features are not independent
- Likely due to mass drift among the profiles
- Weakens the apparent statistical significance of the single feature
- **Loss of correlation among features as a biomarker**?



PEAK	# Features	SN	SP	Test Error
No	5	0.8697	0.669	**0.2132**
No	10	0.9073	0.9039	**0.0941**
No	15	0.9298	0.9537	**0.0603**
No	20	0.9499	0.9502	**0.05**
Yes	5	0.9123	0.726	**0.1647**
Yes	10	0.9424	0.9644	**0.0485**
Yes	15	0.9474	0.968	**0.0441**
Yes	20	0.9348	0.9751	**0.0485**

Risk of cancer	Specificity/Sensitivity	Years gained
5%	0.80	0.11
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$$p(cancer|biomarker) = p(cancer)\ \frac{p(biomarker|cancer)}{p(biomarker)}$$

$$p(cancer|biomarker) = p(cancer)\ \frac{p(biomarker|cancer)}{p(cancer)p(biomarker|cancer)+p(cancer)p(biomarker|cancer)}$$

$$p(cancer|biomarker) = prior\ \frac{sensitivity}{(prior)(sensitivity)+(1-prior)(1-specificity)}$$

Using prevalence = 0.008 as the prior,

Equations derived from Alan Schwartz, UIC
Medical Decision Making, MHPE 494, Spring 1999
http://www.sjdm.org/~alansz/courses/mhpe494/week3.html

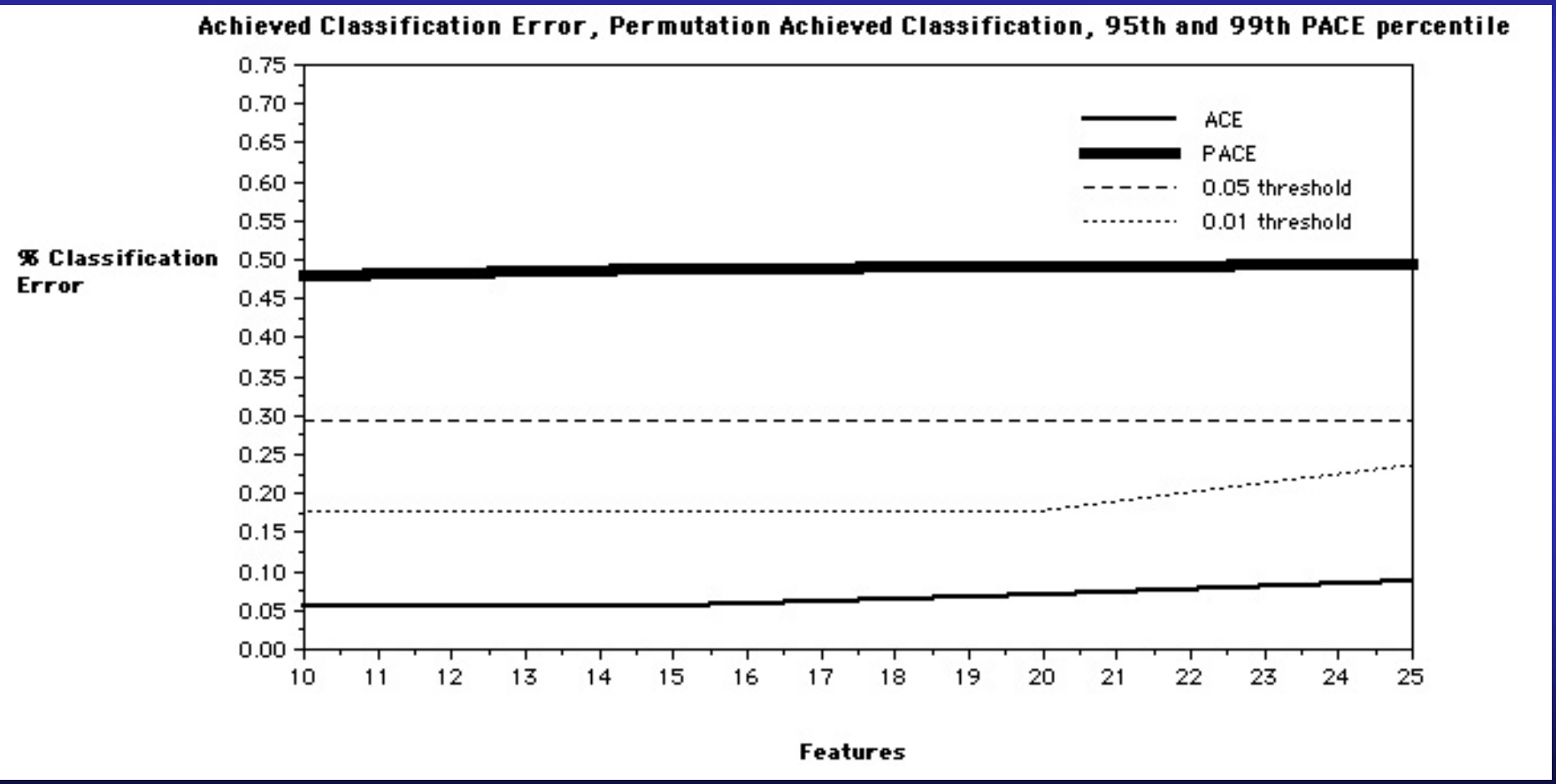
marker	SN	SP	expected false alarms/100k	Reference
invasive techniques (biospy)				
MUC-1 expression (patients w/masses)	0.96	0.94	5995	Chhieng et al., 2003
cytology	0.63	1	0	Mu et al., 2003
p53 expression	0.44	0.8	19992	Mu et al., 2003
cytology + p53	0.78	1	0	Mu et al., 2003
cytology + CA19.9	0.67	0.8	19989	Mu et al., 2003
cytology + p53 +CA19.9	0.78	0.8	19987	Mu et al., 2003
EUS-FNA	0.843	0.97	2997	Eloubeidi et al., 2003
k-Ras mutations	0.27	1	0	Castells et al., 1999
minimally invasive (serum or circulating DNA)				
CA 242 in serum	0.75	0.855	14491	Ozkan et al., 2003
CA 19-9 in serum	0.8	0.675	32476	Ozkan et al., 2003
CEA in serum	0.4	0.73	26991	Ozkan et al., 2003
k-Ras mutations	0.27	1	0	Castells et al., 1999
k-Ras2 mutations	0.46	0.87	12995	Maire et al., 2002
SELDI-TOF-MS SVM+F (overall classifier)	**0.95**	**0.94**	**5995**	**this study****
SELDI-TOF-MS SVM+PCA (overall classifier)	**0.9348**	**0.9751**	**2488**	**this study****

SVM = support vector machine

F = Fisher-score selected features

PCA = principal-component selected features

Preliminary Pancreatic Cancer Result

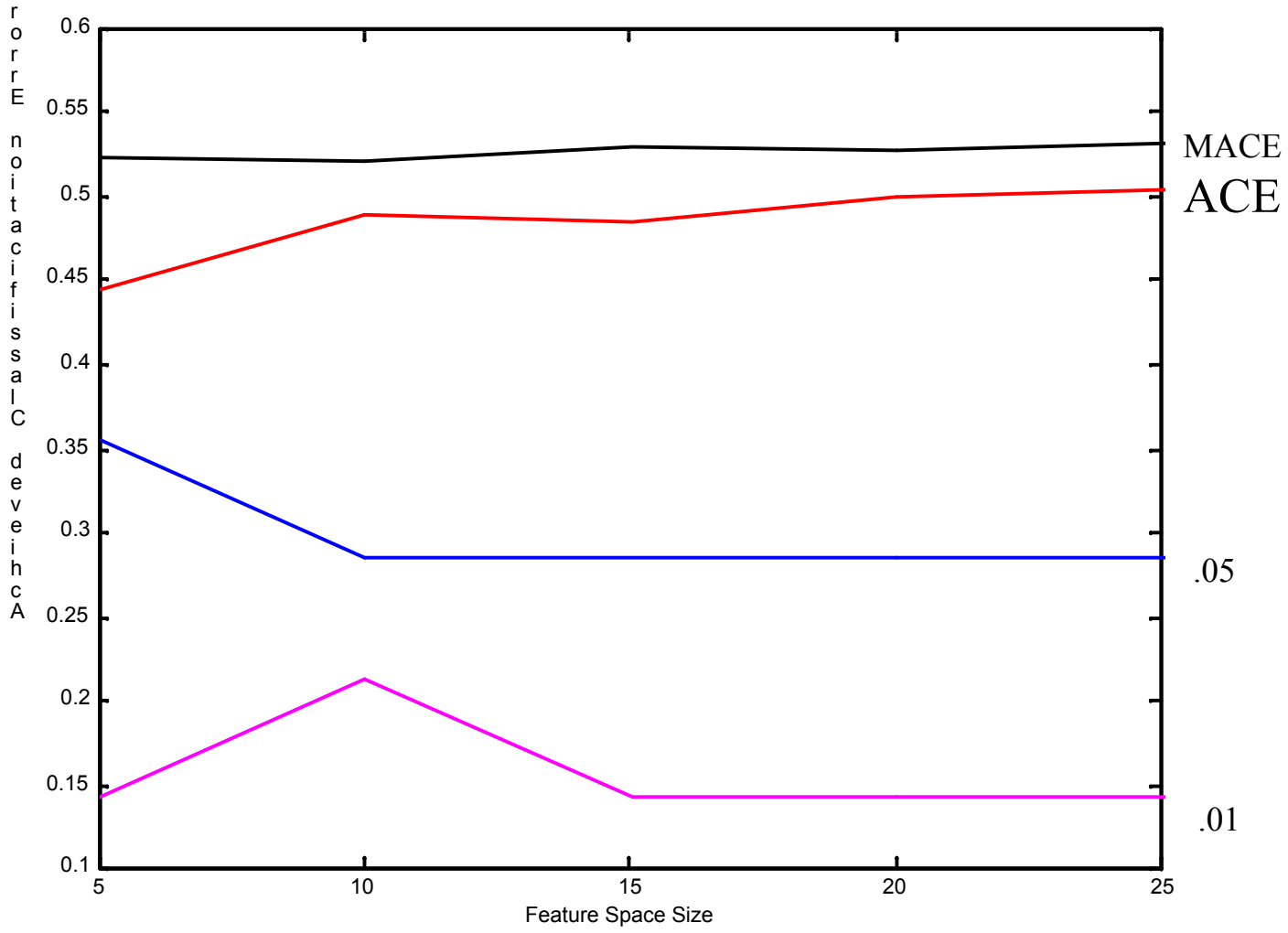


I have a statistical basis for confidence…

- *Yea, though I walk through the valley of the shadow of death, I will fear no evil*

Much more is at stake than our careers

- *Be concerned about patient care*
- *Errors in either direction (overly optimistic or overly pessimistic) could be costly.*



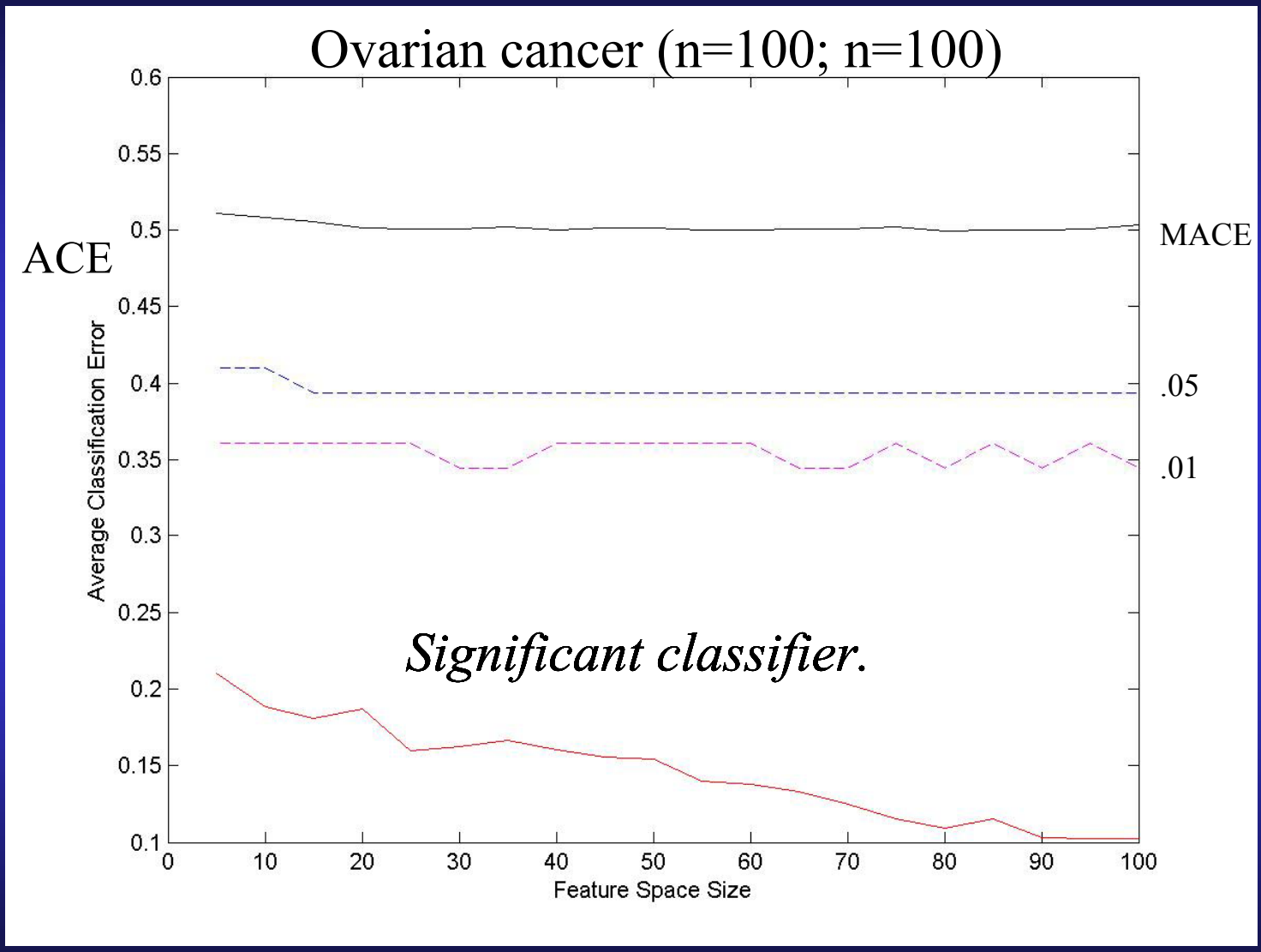
AUC, No Decorr, Naïve Bayes

Reason for No Significance?

- No biological signal
- Poor study design
- Need for improved technology
- Weak signal (insufficient samples, power of the test)

What of Ovarian Cancer?

Use of proteomic patterns in serum to identify ovarian cancer.
Lancet. 2002 Feb 16;359(9306):572-7.


Ovarian cancer (n=100; n=100)
ACE
MACE
.05
.01
Significant classifier.
Average Classification Error
Feature Space Size
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0
10
20
30
40
50
60
70
80
90
100

SVM + PCA

Claim, Conjecture, or Tautology?

- Peform methods $x_1, x_2, x_3 \ldots x_n$
- Which ever steps in any method reduce ACE are not helpful
- Which ever method(s) exhibit significant ACE are justified
- Which ever methods achieve lowest ACE is preferred
- Different features identified by different methods w/significant ACE are all interesting

What about your SOP's?

- Could PACE be used to optimize laboratory protocols?
- Could PACE be used to optimize preprocessing?
- Could PACE be used to optimize analysis strategies?

Caveats

- PACE **does not** protect against improper study design (e.g., confounding)
- Signal detected is tentative and requires
  - (a) evaluation of classifier with new, unseen samples
  - (b) replication of the study*
  - (c) identification of peptide complexes
- Ultimate validation derives from shift in the proportion of cases detected at earlier stages and increases in survivorship.

Biological vs. Statistical Significance

Universal Biomarker Myth

- Each tumor is a unique biological event
- "Cancer" often includes various stages
- Unique genes/proteins may be lost in each patient
- Patient classes my be unique to genes (this is not merely disease subclasses)
- Proportion of samples in which a feature is informative
- Biomarker Panels:
  - Not protein 1 AND protein 2 AND protein 3…*N*
  - Protein 1 OR 2 OR 3…*N*
- Accommodate Individualized Medicine

Biological vs. Statistical Significance 2: Systematic Juvenile Rheumatoid Arthritis

- Raphael Hirsch
- Takako Miyamae
- Shumpei Yokota
- Bonnie Lemster
- David Malehorn
- Bill Bigbee

Please contact Dr. Hirsch

Required Reading

- Mehta AI, Ross S, Lowenthal MS, Fusaro V, Fishman DA, Petricoin EF 3rd, Liotta LA. Biomarker amplification by serum carrier protein binding. Dis Markers. 2003-2004;19(1):1-10.

Open Areas

(1) Why Peaks Only?

(2) Workflow/Protocol Optimization

Peaks Etc?

- Peaks (Directed Approach)
- Troughs (Directed Approach)
- Slopes (Directed Approach)
- Binning (Partially Directed Approach)
- Whole-Profile Analysis (Non-directed Approach)
- Anti-Peaks (All m/z values - peaks) (Anti-directed Approach)

- Which one has more information?

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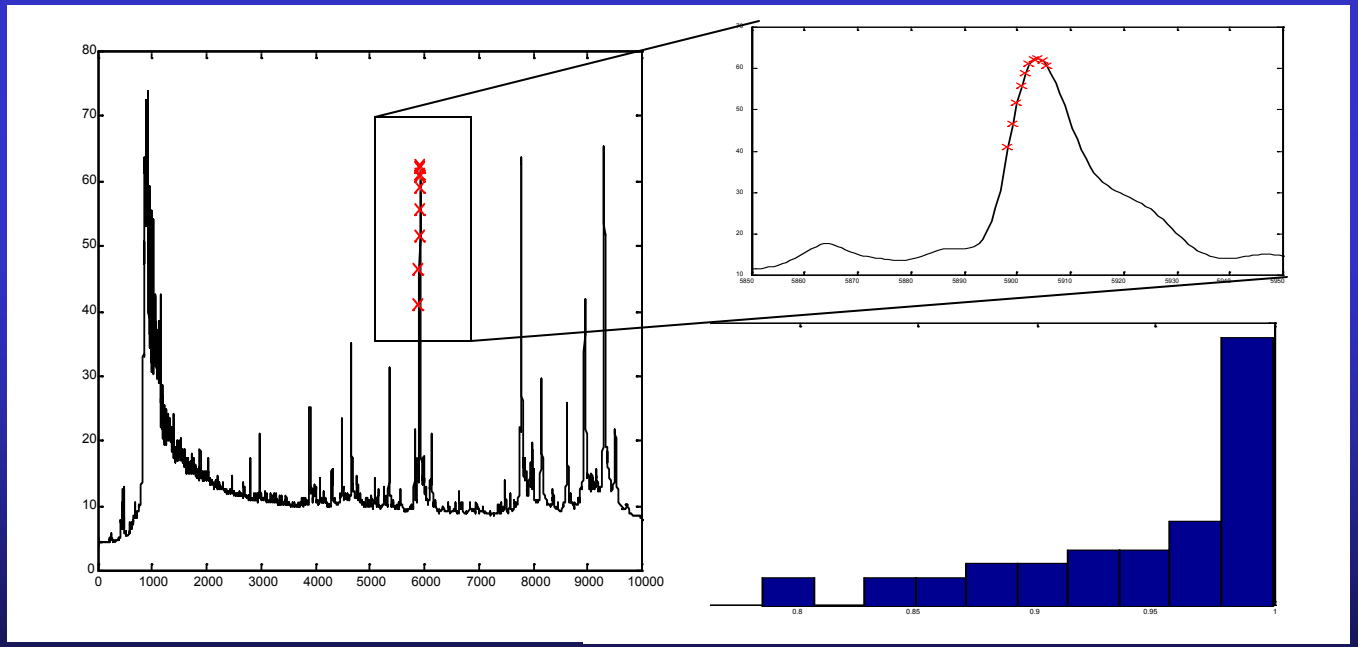
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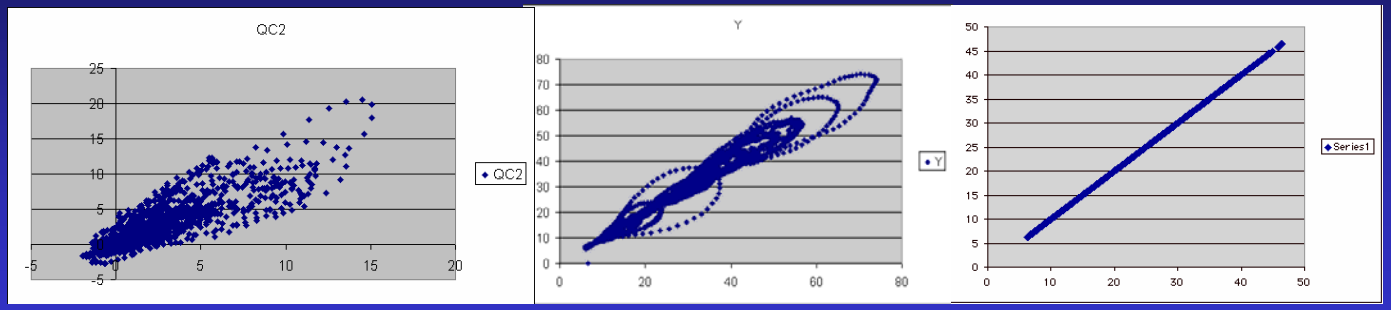
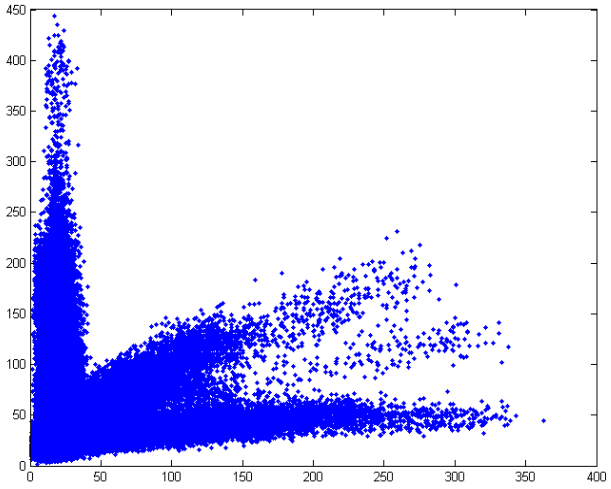
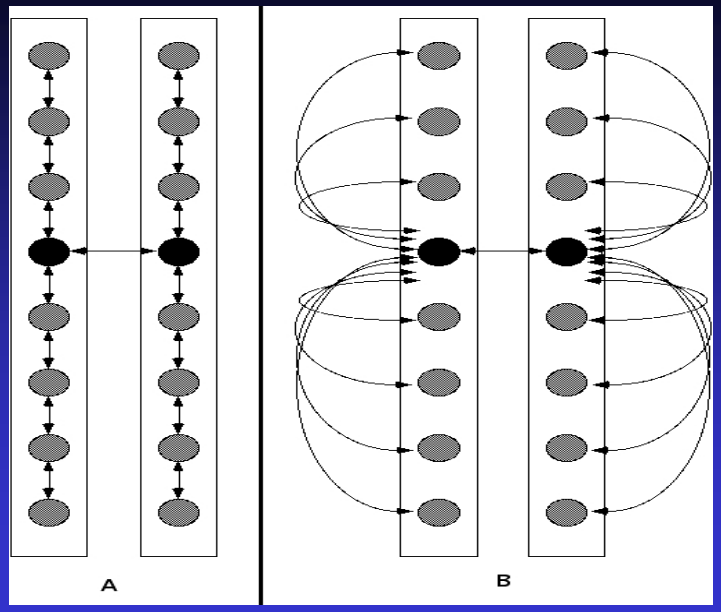
Mass Drift as Seen in Pooled Reference Serum



Samples on Q*

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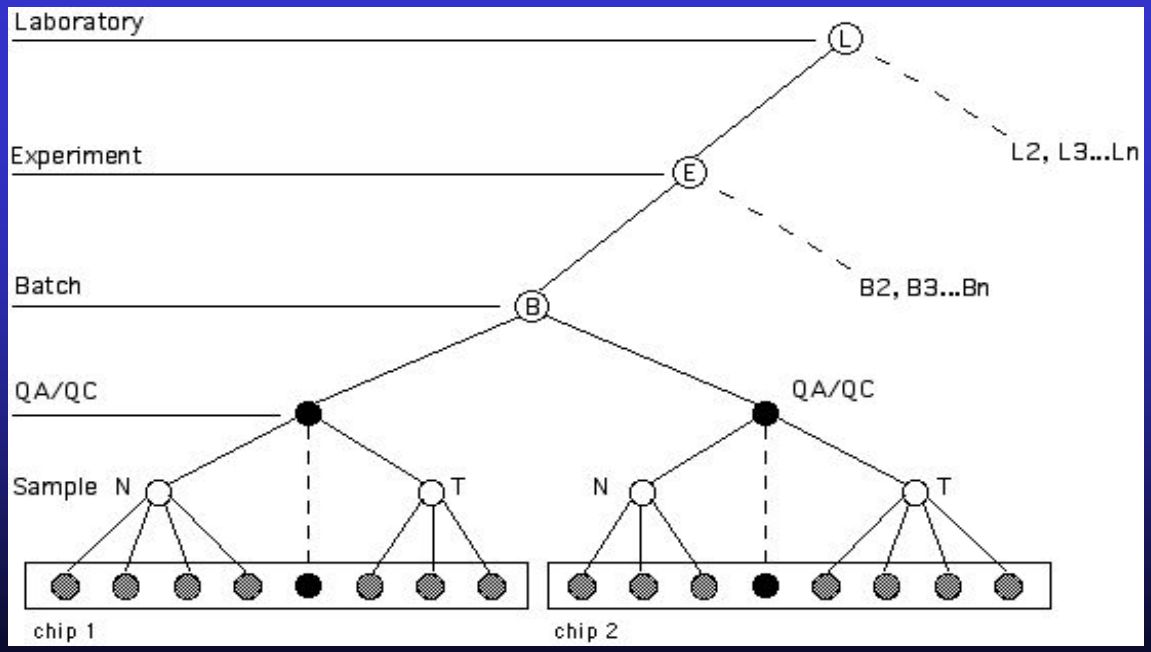
B


Laboratory
L
L2, L3...Ln
Experiment
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B2, B3...Bn
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QA/QC
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Open Areas

(1) Why Peaks Only?
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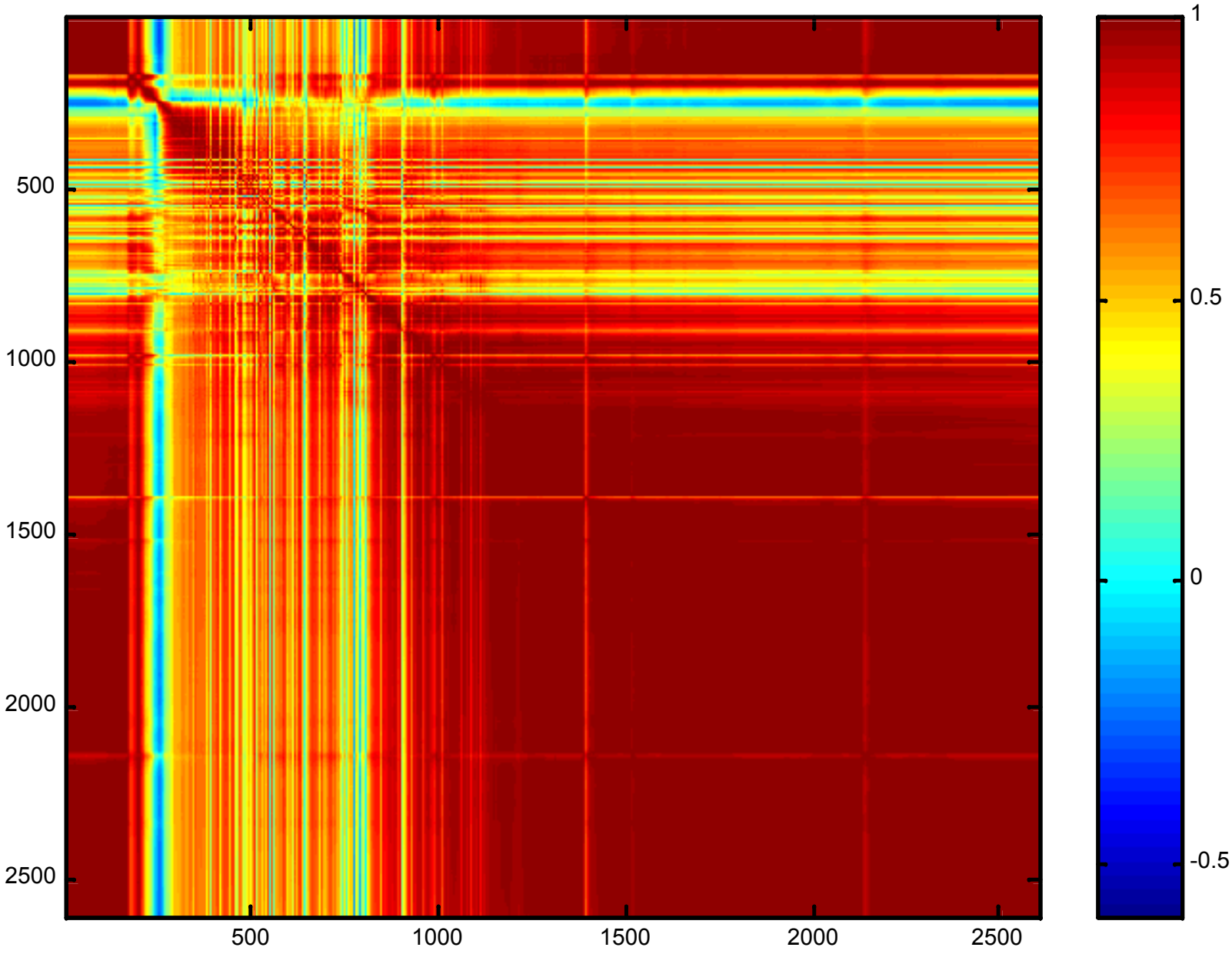
(3) Profile alignment/mass calibration+
(4) Anti-correlated features as cancer biomarkers?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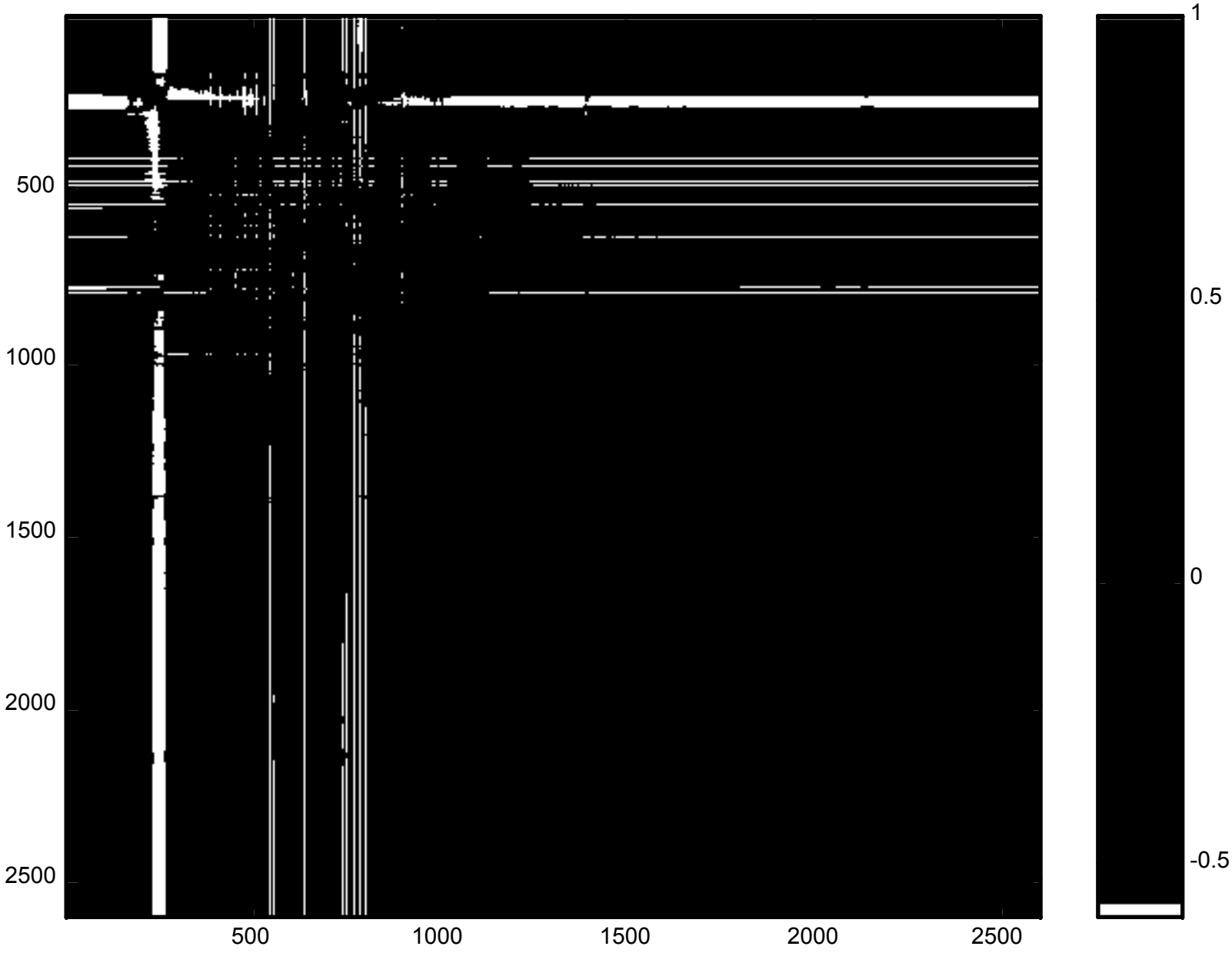
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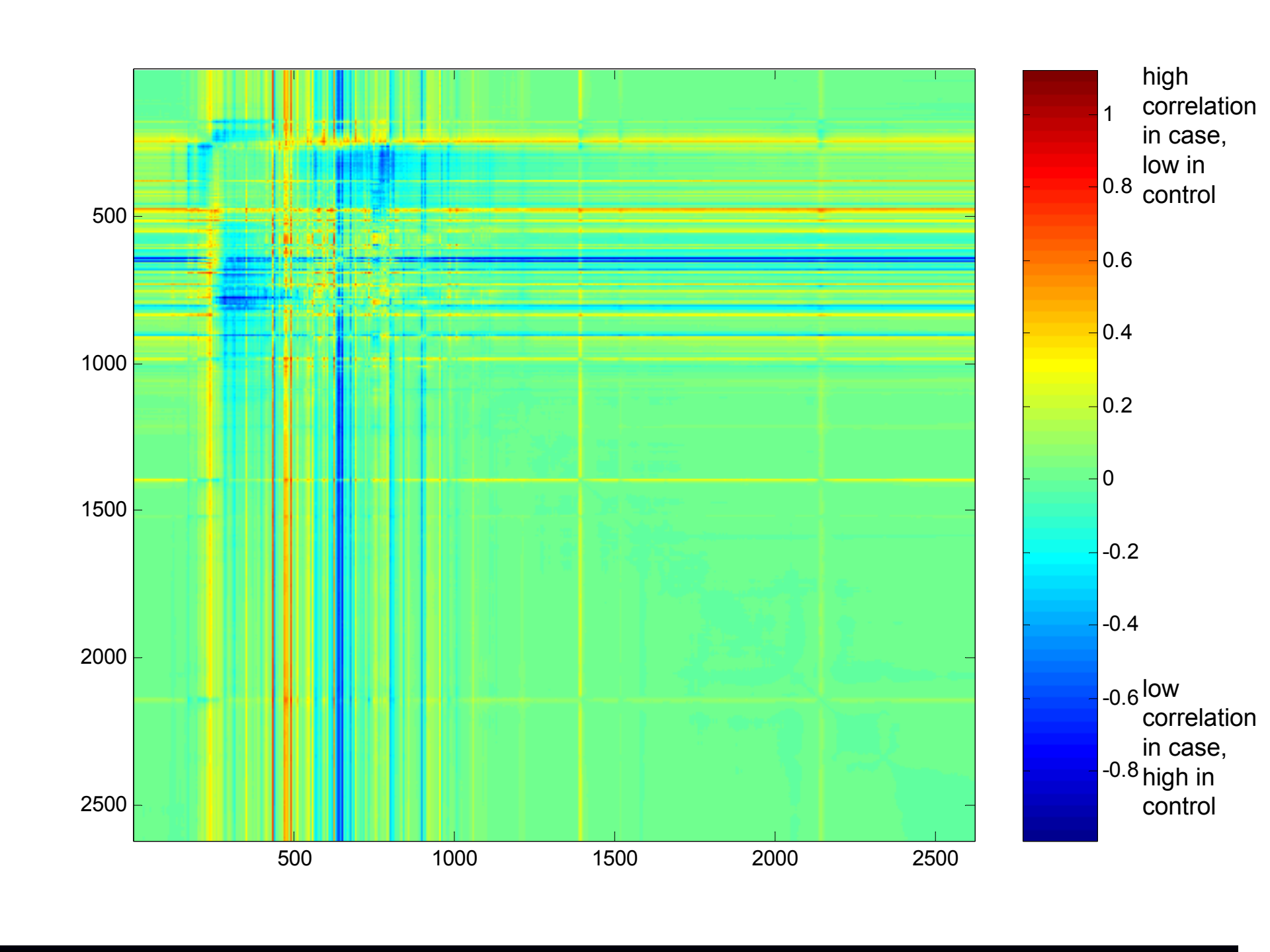
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500
1000
1500
2000
2500
high correlation in case, low in control
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0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
low correlation in case, high in control

Open Areas

(1) Why Peaks Only?
(2) Workflow/Protocol Optimization
(3) Are the markers cancer-specific?
(4) Profile Alignment (mass calibration) +
(5) Anti-correlated features as cancer biomarkers?
(6) Relating Cellular Proteome to the Transcriptome

Open Areas

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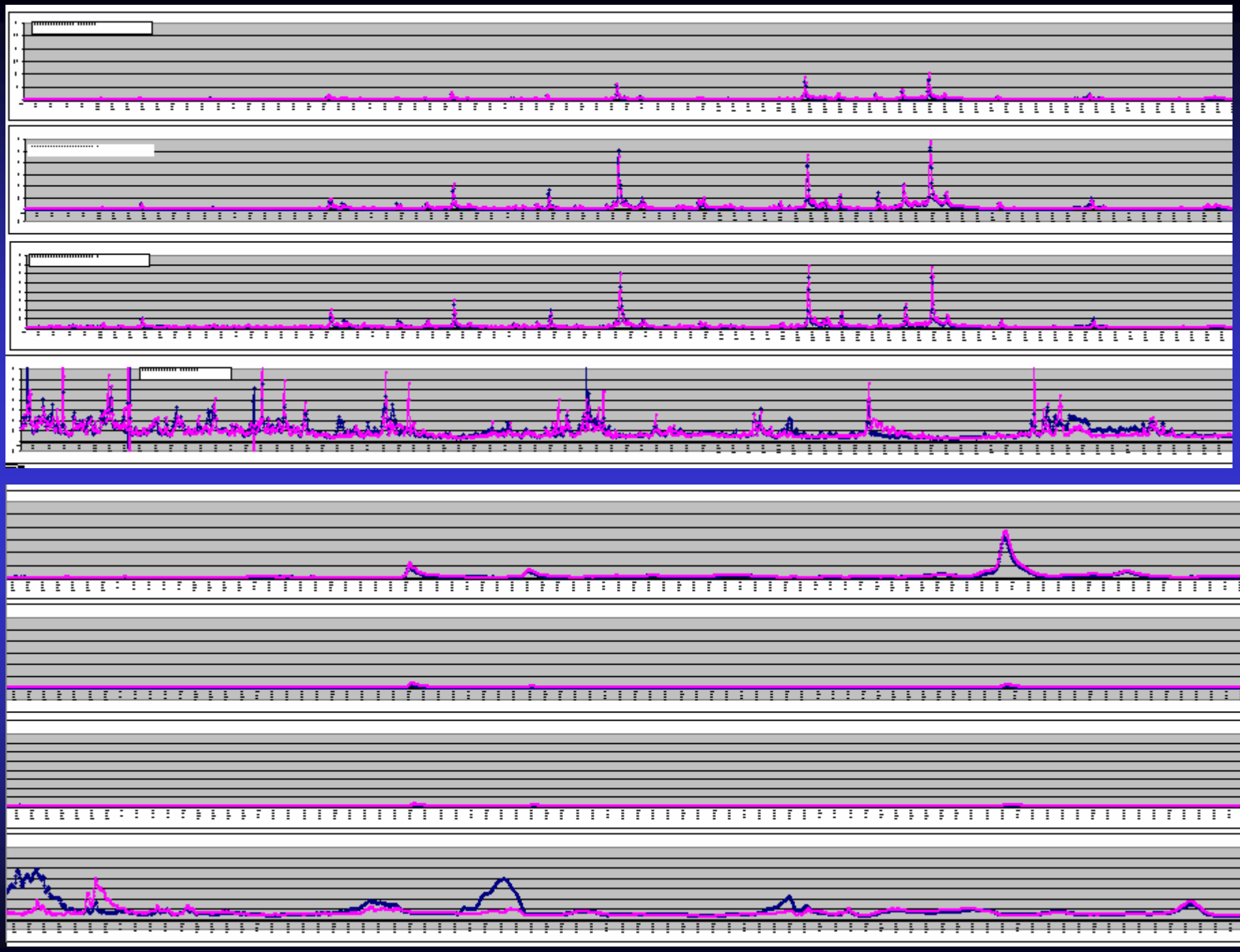
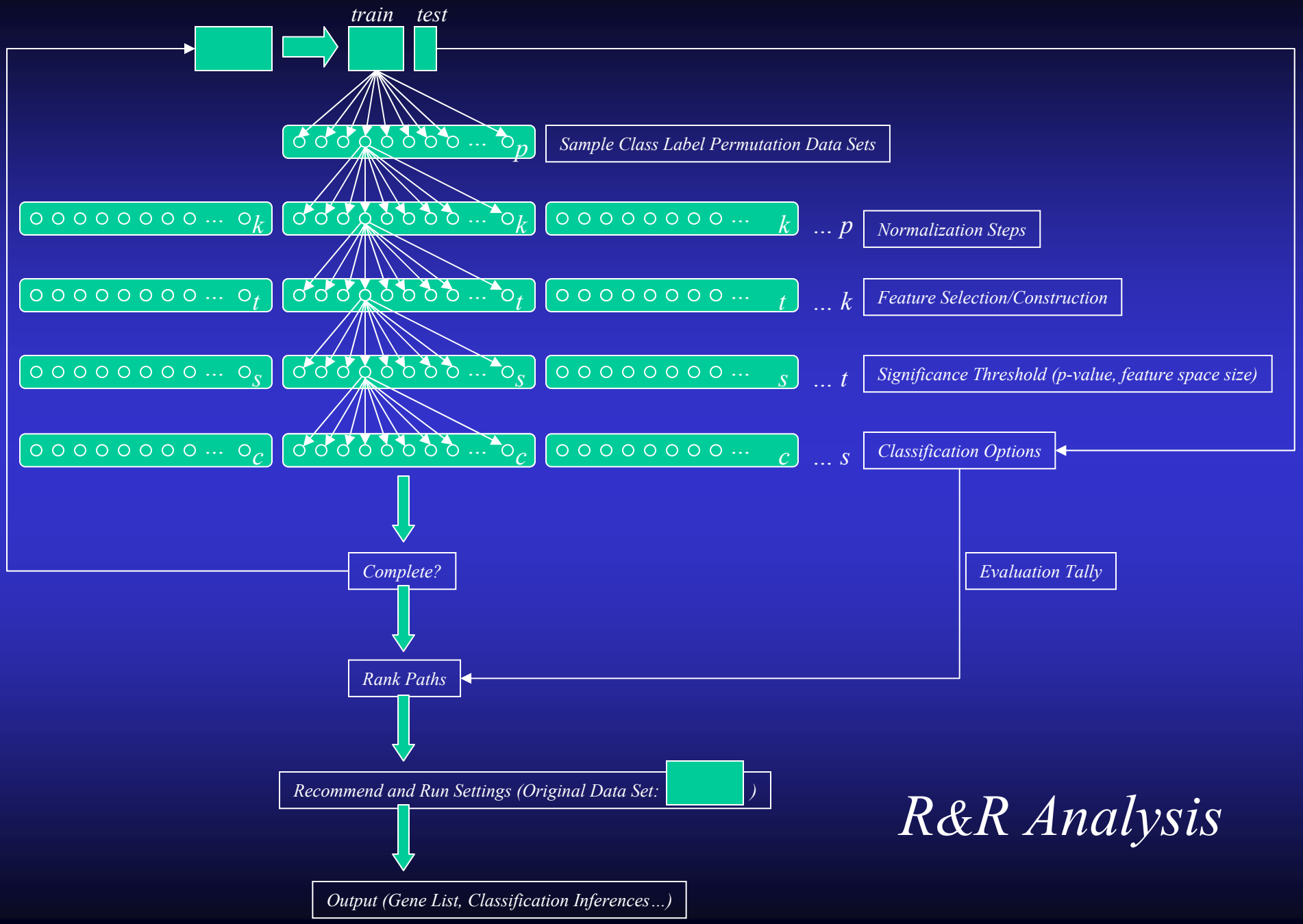
(4) Profile Alignment (mass calibration) +
(5) Anti-correlated features as cancer biomarkers?
(6) Relating Cellular Proteome to the Transcriptome
(7) Permutation-based feature selection
(8) Network (grid) of Computational Clusters Dedicated to HTP Proteomic Analysis




train
test
p
Sample Class Label Permutation Data Sets
k
k
k
… p
Normalization Steps
t
t
t
… k
Feature Selection/Construction
s
s
s
… t
Significance Threshold (p-value, feature space size)
c
c
c
… s
Classification Options
Complete?
Evaluation Tally
Rank Paths
Recommend and Run Settings (Original Data Set:)
Output (Gene List, Classification Inferences…)

R&R Analysis

CARRAY

The Gene Expression Data Portal
GEDP.nci.nih.gov

CMAP

MatchMiner

BIOgopher POWERED BY caBIO

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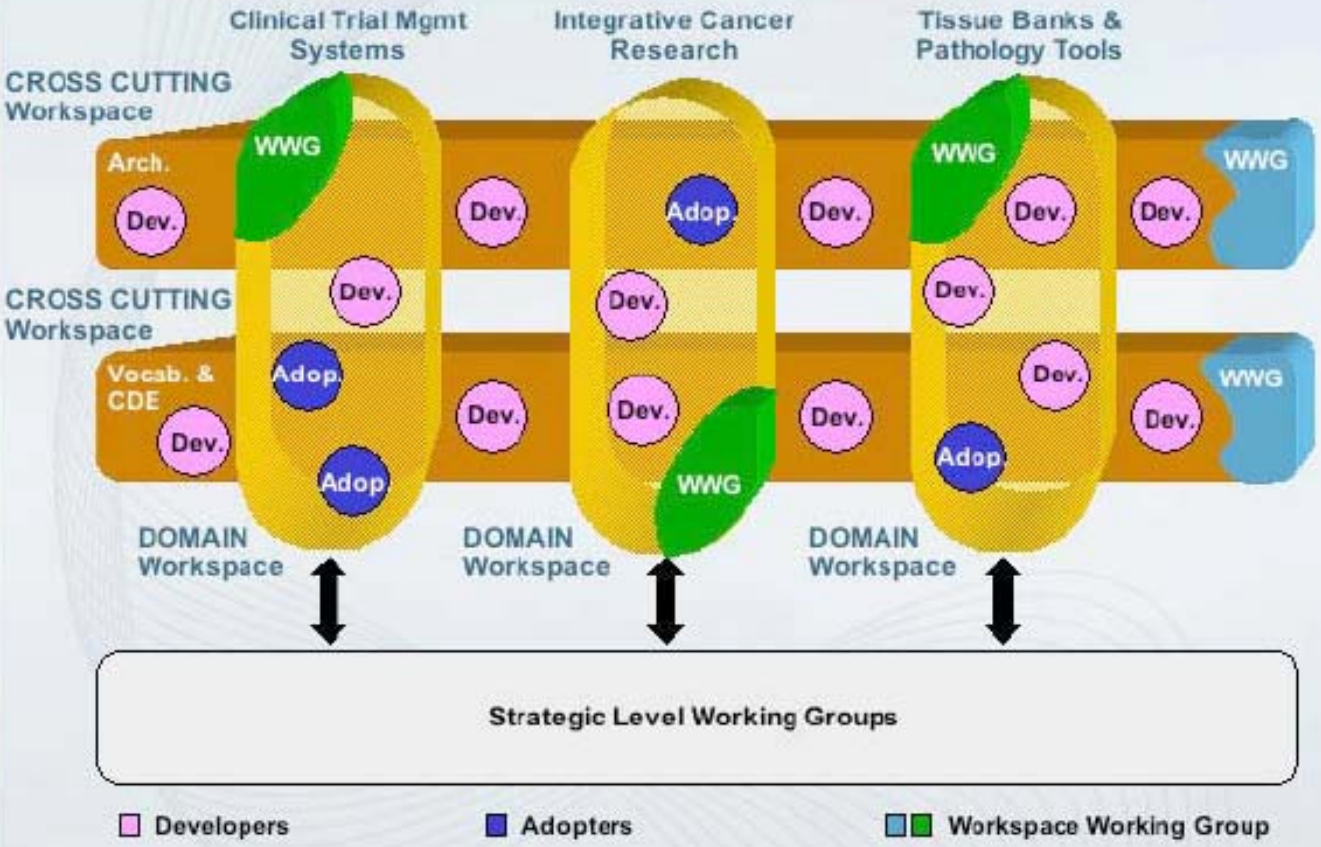
n

caBIG Facts and activities – Structure

The overall structure of the caBIG pilot is shown in the diagram below. An overview of these elements is given subsequent to the diagram.



http://cabig.nci.nih.gov/

caBIO

caDSR

EVS

Thing (ca) BIG!

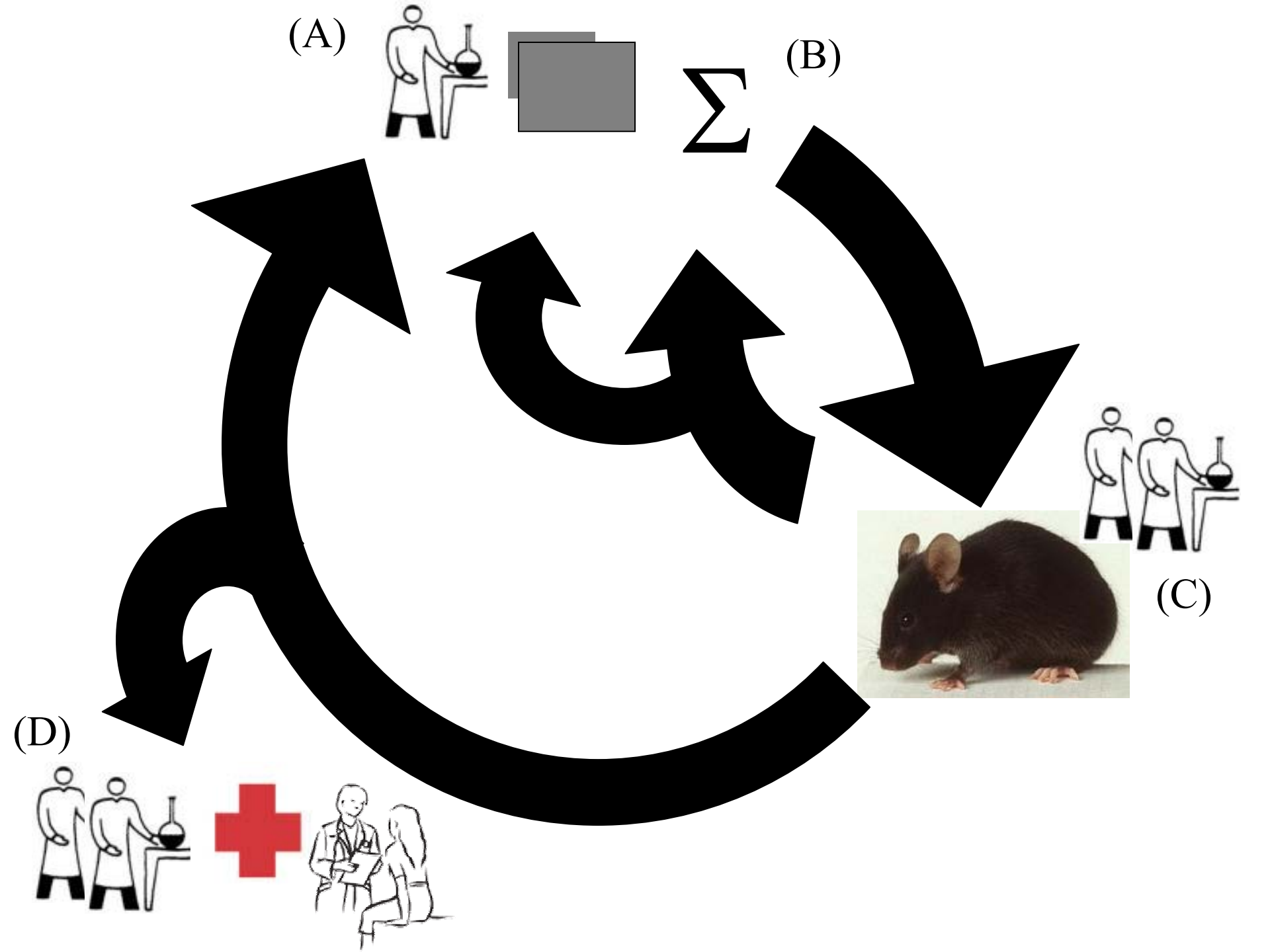
- Open Source
- Open Access
- Standardized Development
- Grid Computing?
- Develop & Participate
- National Integrative Cancer Research Databases

Open Areas

(1) Why Peaks?
(2) Workflow/Protocol Optimization
(3) Are these markers cancer-specific?
(4) Profile Alignment (mass calibration)+
(5) Look for anti-correlated features as cancer biomarkers
(6) Relating Cellular Proteome to the Transcriptome
(7) Permutation-based feature selection
(8) Network (grid) of Computational Clusters Dedicated to HTP Proteomic Analysis

Conclusions - and a Challenge

- Room for Improvement
- We recommend that all results achieved with new data sets - and prior results achieved with published data sets - be evaluated with PACE.


(A)
(B)
(C)
(D)

Turns of the Cycle (Future Applications)

(1) Cancer Detection

(2) PROGRESSION/Identification of Targets

(3) Recurrence

(4) Theranosis (Prediction of Therapy Outcome)

(5) Therapy Dose/Scheduling (real-time)

(6) Unraveling the immunological stories…

CPRN?

(1) Encourage and enable studies of the mechanisms of cancer PROGRESSION

(2) Re-analysis of published microarray and SELDI/MALDI-TOF data sets

(3) Identification and characterization of PROGRESSION Targets

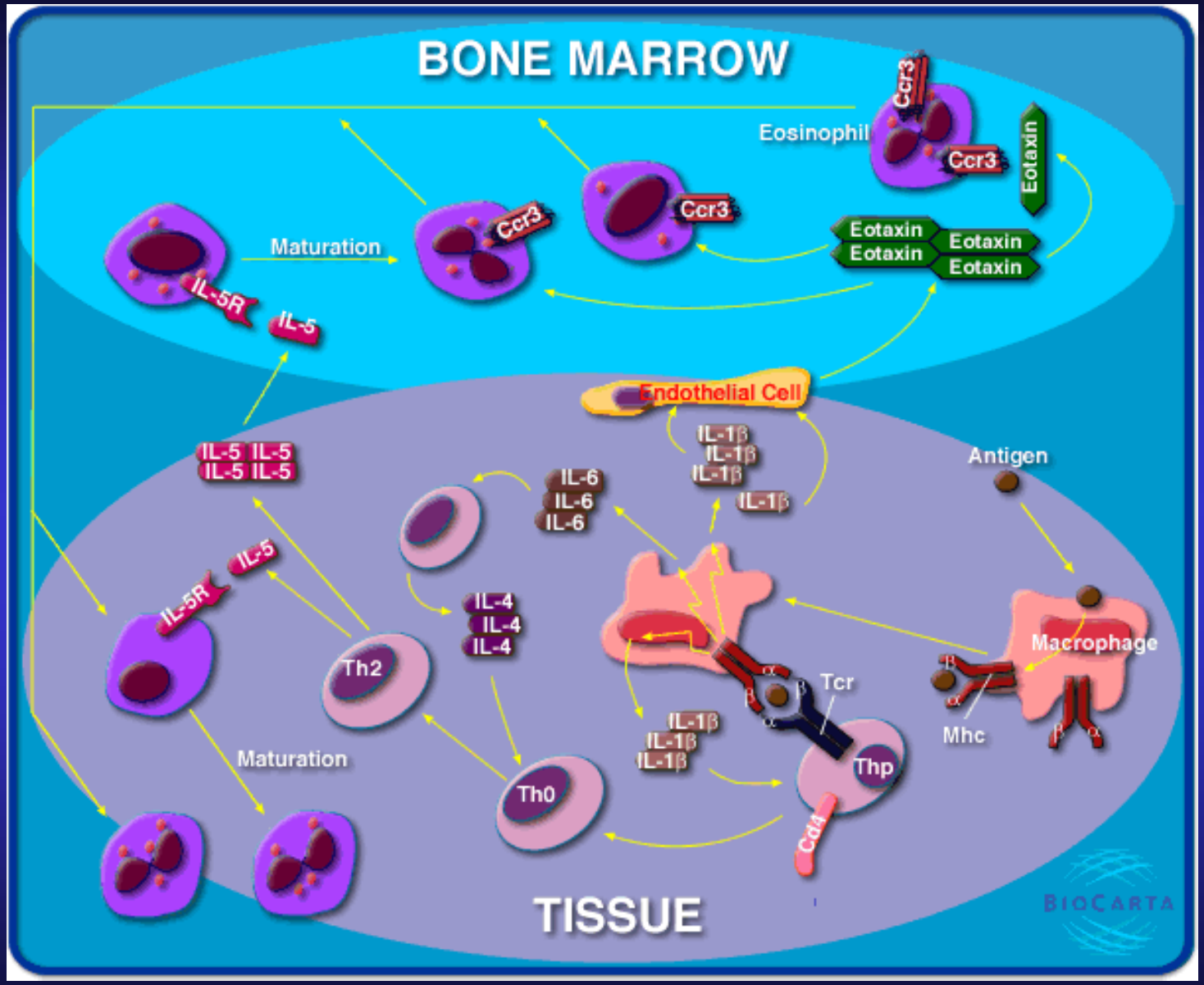
(4) Include invitations to primary authors on original studies

(5) Animal Model Focus

(6) Lay up for Clinical Trials

Example: Astrocytoma

- 3 Published Data Sets
- Fairly poor analysis
- Goldmine for consumers
- 18 genes differentially expressed in 2 data sets
- 8 genes differentially expressed in 3 data sets
- These are now validated progression markers.
- So?


BONE MARROW
Eosinophil
Ccr3
Ccr3
Eotaxin
Ccr3
Ccr3
Maturation
Eotaxin
Eotaxin
Eotaxin
Eotaxin
IL-5R
IL-5
Endothelial Cell
IL-1β
IL-1β
IL-1β
IL-1β
Antigen
IL-5 IL-5
IL-5 IL-5
IL-6
IL-6
IL-6
IL-5
IL-5R
IL-4
IL-4
IL-4
Th2
Macrophage
Tcr
IL-1β
IL-1β
IL-1β
Mhc
Thp
Maturation
Th0
Cd4
TISSUE
BioCarta

- There are fewer producers than consumers - not for long.
- Learn Slowly
- Understand fundamental characteristics of the data
  - Lab worflows, sources of variability
  - Statistical Properties
- Communicate clearly
  - With each other
  - With our clients!
- Focus on clinical translation of knowledge acquired by our efforts.
- Effort to ID peptides at each m/z value detected by each chip

Feb 13, 2003 Ciphergen Reports Record Financial Results for the Fourth Quarter and Fiscal Year 2002

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http://apiii.upmc.edu/

Applied Bioinformatics

EDRN themed volume, late 2004/early 2005

Please submit additional articles as well!

(lyonsweilerj@upmc.edu)